AWARD NUMBER: W81XWH-16-1-0288

TITLE: A Novel Pleiotropic Anti-Inflammatory Drug to Reduce ARDS Incidence

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REPORT DATE: July 2017

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
July 2017	Annual	1 Jul 2016 - 30 Jun 2017

4. TITLE AND SUBTITLE A

A Novel Pleiotropic Anti-Inflammatory Drug to Reduce ARDS Incidence

5a. CONTRACT NUMBER

5b. GRANT NUMBER W81XWH-16-1-0288

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Upstate Medical University
Syracuse, NY 13210-2375

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
Our trauma/hemorrhagic shock (T/HS) injury model was highly effective at causing acute respiratory distress syndrome (ARDS) in all Control groups. However, TRB-N0224 treatment, although it lowered both plasma and bronchoalveolar lavage (BALF) IL-6 levels, resulted in no significant improvement in clinical outcome, in the form of improve lung function (i.e lung compliance or PaO_2/FiO_2 ratio) or histopathology. We postulate that there were two problems with the study: 1) the stress of the gavage was an additional trauma in an already severe T/HS model and 2) the T/HS model causes severe damage to the gut, which significantly reduced TRB-N0224 adsorption. To solve this problem we have requested a one-year no-cost extension to use an intravenous formulation of TRB-N0224 that, if our postulate is correct, will solve both of our problems. As a secondary solution to our problem if the IV formulation of TRB-N0224 is not effective in the T/HS model we will try in our rat cecal ligation and puncture (CLP) model. This will determine if TRB-N0224 is effective for trauma and/or sepsis.

15. SUBJECT TERMS Hemorrhagic shock, acute respiratory distress syndrome (ARDS), TRB-N0224 to prevent ARDS, anti-inflammatory

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified UU	6	19b. TELEPHONE NUMBER *(include area code)*

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Introduction

The goal of this study was to determine the proof of concept that the pleiotropic anti-inflammatory drug, TRB-N0224, was effective at reducing the development of the acute respiratory distress syndrome (ARDS) in a rat trauma/hemorrhagic shock (T/HS) model.

Keywords

Acute respiratory distress syndrome, ARDS, systemic inflammatory response syndrome, SIRS, matrix metalloproteinases, MMP, curcumin, TRB-N0224, cytokines

Accomplishments

What were the major goals of the project: The goal of this study was to determine the proof of concept that the pleiotropic anti-inflammatory drug, TRB-N0224, was effective at reducing the development of the acute respiratory distress syndrome (ARDS) in a rat trauma/hemorrhagic shock (T/HS) model.

What was accomplished under these goals: IACUC approval was obtained (month 2) and TRB-N0224 was formulated for use and sent to our lab to begin this study (month 5). We began conducting the experiments in the Control groups to confirm that our T/HS model resulted in multiple organ failure, including acute respiratory distress syndrome (ARDS) (months 6-8). Our data clearly showed that T/HS caused ARDS as measured by the fall in PaO_2/FiO_2 ratio and in lung compliance (Fig 1 A,B). Qualitative lung histopathology confirmed that animals had developed an acute lung injury. We discussed with our co-investigator at Stony Brook (Dr. Lorne Golub) what he postulated to be the optimal treatment regimen. The reason for beginning with what we felt was the optimal treatment strategy, was to ensure that the T/HS model was not moribund such that no treatment would work. Dr Golub suggested a 7-day TRB-N0224 pretreatment regimen of by oral gavage (30mg/kg). Experiments with this treatment strategy were conducted in months 8-11. Unfortunately, we did not measure a physiologic/clinical treatment effect with our postulated optimal treatment strategy (Fig 1A,B). In month 17 we analyzed the plasma and bronchoalveolar lavage fluid (BALF) for the inflammatory cytokine IL-6 and showed that TRB-N0224 resulted in a decrease in IL-6 in both plasma and BALF. We discussed and analyzed the data in month 12 and decided the optimal strategy would be to modify the route of drug delivery from gavage to intravenous (IV). We tested several vehicles and decided to go with an ethanol base vehicle, since TRB-N0224 is not water-soluble. We decided to hold off on the other molecular assays and extensive histopathologic analysis until we have identified the optimal vehicle and dose for TRB-N0224. We have requested a 1yr no-cost extension to conduct the IV experiments.

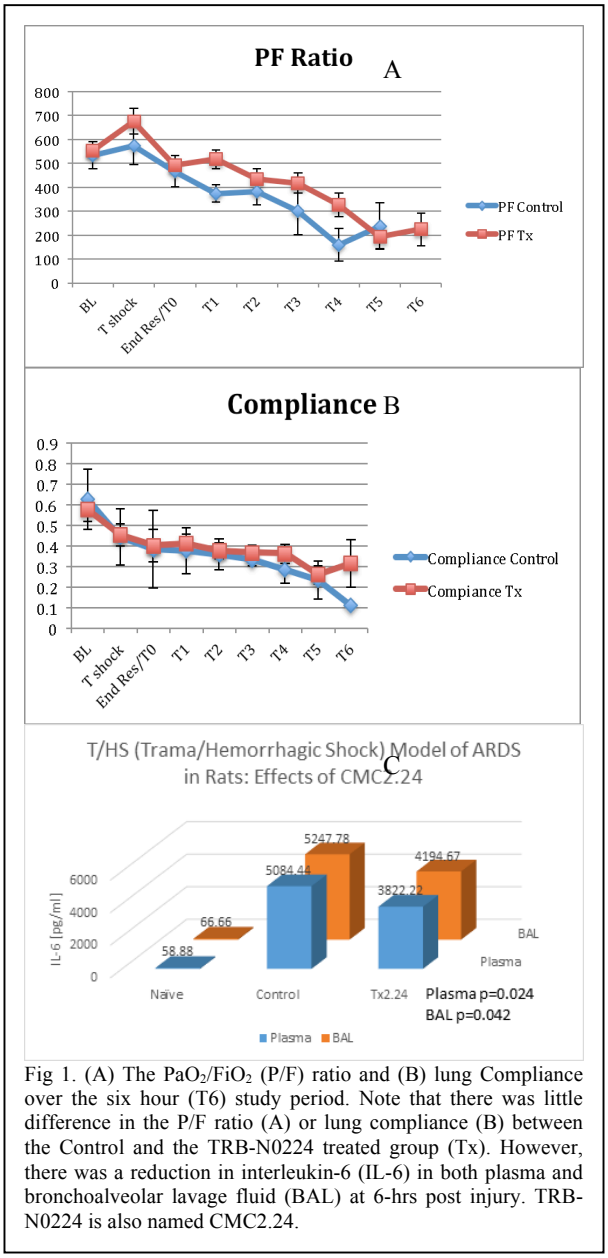

Fig 1. (A) The PaO_2/FiO_2 (P/F) ratio and (B) lung Compliance over the six hour (T6) study period. Note that there was little difference in the P/F ratio (A) or lung compliance (B) between the Control and the TRB-N0224 treated group (Tx). However, there was a reduction in interleukin-6 (IL-6) in both plasma and bronchoalveolar lavage fluid (BAL) at 6-hrs post injury. TRB-N0224 is also named CMC2.24.

What opportunities for training and professional development: Nothing to report.

How were the results disseminated to communities of interest: Nothing to report

Impact

What was the impact on development of principal discipline(s) of the project: Nothing to report.

What was the impact on other disciplines: Nothing to report.

What was the impact on technology transfer: Nothing to report.

What was the impact on society beyond science and technology: Nothing to report

Changes/Problems

Changes in approach and reasons for change: Although we saw a positive impact of TRB-N0224 on inflammatory mediators (Fig 1C) we did not reduce the incidence of the acute respiratory distress syndrome (ARDS), which was our primary goal. We postulate that the most likely reason for these negative results was either our injury model is too severe causing the animals to be moribund or the mode of drug delivery (i.e. gavage) was not effective at delivering a pharmacologically effective blood concentration of drug.

Actual or anticipated problems or delays and actions or plans to resolve them: We believe that our general approach in this study remains solid. We have requested a 1yr no-cost extension to test our hypothesis that intravenous (IV) delivery of TRB-N0224 will significantly improve the protective effect on the lung. If IV therapy fails in the current injury model we will decrease the severity of the injury model and repeat the study.

Changes that had a significant impact on expenditures: We did not complete all treatment groups because it would be fruitless to test smaller doses if the optimal dose failed to reduce ARDS incidence. Also we did not run all of the molecular assays for the same reasons and thus we have sufficient funding remaining to test our IV therapy hypothesis in the 1yr no-cost extension. Thus, the changes in our drug delivery strategy will have no impact on expenditures.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents: Nothing to report.

Products: Nothing to report

Participants & Other Collaborating Organizations

Name	Gary Nieman
Project Role	PI
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	2
Contribution to Project	Oversaw all experiments, analyzed and intreprated all results and constructed the Final Report
Funding Support	(Complete only if the funding support is provided from other than this award)

Name	Reasearch Scientist
Project Role	
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	6
Contribution to Project	Performed all surgical proceedures on the rats, conducted the experiments as instructed by the protocol and perfomed the necropsy at the end of each experiment. Collected all data onto Excel spread sheets and ran statistical analyses on these data.
Funding Support	(Complete only if the funding support is provided from other than this award)

Name	Louis Gatto
Project Role	Co-investigator
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	1
Contribution to Project	Histologic analysis of a tissue samples, analyzed and intreprated all results from these experiments
Funding Support	

Name	Lorne Golub
Project Role	Co-investigator
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	1
Contribution to Project	Formulated TRB-N0224, analyzed and intreprated all results from these experiments
Funding Support	(Complete only if the funding support is provided from other than this award)

Name	His-Ming Lee
Project Role	Research Assistant Professor
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	4
Contribution to Project	Dr Lee ran all of the molecular mediator assays
Funding Support	(Complete only if the funding support is provided from other than this award)

Special Reporting Requirements: Nothing to report

Appendices: Nothing to report